REMARKS

It is respectfully requested that this application be reconsidered in view of the above amendments and following remarks; and that all of the claims remaining in this application be allowed. Claims 57-69 are pending in this application.

Summary Of The Invention

Applicants have developed a pioneering invention, i.e., novel chimeric DNA sequences that encode protein receptors for signal transduction in effector host cells in which the proteins are expressed. The chimeric proteins contain a cytoplasmic region of a signal transducing molecule that is covalently joined via a transmembrane domain to the extracellular binding domain of another surface membrane protein or secreted protein which is ligand binding, all in a single protein molecule. Thus, the extracellular domain can now be varied to allow a signal to be transduced in response to binding different ligands, and redirect the host effector cell bearing the chimeric receptor to various ligand targets. Moreover, the extracellular binding domain is not MHC restricted and an antigen presenting cell is not required to activate the host cell.

The Amendments

The amendments made in the pending claims will be discussed in the order that an objection or rejection was raised in the September 19, 1995 Office Action. No new matter has been added by the amendments requested in this response. An information disclosure statement has been filed

simultaneously with the instant responsive amendment. A substitute declaration will be submitted soon to overcome the defective declaration by inventor Bryan Irving. Claim 70 has been canceled without prejudice because it claimed identical subject matter set forth in claim 69.

Rejection Under 35 U.S.C. 112, First Paragraph

Claims 57-69 stand rejected under 35 U.S.C. 112, first paragraph, alleging that the disclosure is enabling only for claims limited to chimeric proteins as set forth by the Examiner on pages 3-5 of the Office Action. This rejection is traversed for the reasons set forth below.

Claim 57 has been amended to now recite the functional language that the membrane bound chimeric protein will initiate signaling when the extracellular binding domain of the chimeric protein binds to a ligand. Claim 57 has also been amended such that the host cell is now recited to be a mammalian cell. It is submitted that claim 57 as now amended more accurately states the functional properties of the chimeric protein claimed in the instant invention.

Claim 57 recites that the cytoplasmic domain of the novel chimeric receptor activates a messenger system upon ligand binding to the extracellular binding domain. The Examiner contends that the specification does not appear to describe the metes and bounds of messenger systems other than a secondary messenger systems. This rejection is traversed.

Applicants draw the Examiner's attention to page 2, line 5 to line 16 of the instant specification: "In some instances, the change in the cytoplasmic portion results in binding to other proteins, where the other proteins are activated and may carry out various functions. In some situations, the

cytoplasmic portion is autophosphorylated or phosphorylated resulting in a change in its activity. These events are frequently coupled with secondary messengers, such as calcium, cyclic adenosine monophosphate, inositol phosphate, diacylglycerol, and the like. The binding of the ligand results in a particular signal being induced." Applicants submit that it is well established in the field that the term "messenger system" includes all of the above recited components and not merely the secondary messengers listed above. The law is clear on this matter. The claimed subject matter need not be described *in haec verba* (in the same words) to satisfy the description requirement. In re Smith, 59 CCPA 1025, 458 F.2d 1389, 173 USPQ 679 (1972). It is not necessary that the application describe the claimed limitation exactly, but only so clearly that one having ordinary skill in the pertinent art would recognize from the disclosure that Applicants invention includes those limitations. In re Smythe, 480 F.2d 1376, 178 USPQ 279 (CCPA 1973).

Regarding page 3 of the Office Action, second paragraph, Applicants point out that claim 57 and dependent claims therefrom have now been amended to clarify the breadth of the claimed invention. The novel chimeric protein and the host cells that contain them as now recited in the claims requires that the "membrane bound protein initiates signaling in said host cell when the extracellular binding domain binds to said ligand." The specification points out how to use the chimeric proteins that initiate signaling in a host cell. Accordingly, the breadth of the instant claims is clearly set forth and enabled because all of the chimeric receptors encompassed by the claimed inventions must be able to initiate a signal in a mammalian host cell upon binding to ligand.

The Office Action cites the Stoddard et al reference to reject the breadth and scope of the claims recited in the instant invention. This reference describes theoretical mechanisms for transmembrane signaling. For the reasons set forth below, Applicants contend that this reference does not apply to the present invention. In the first place, Models A, B and C from Stoddard et al refer to mechanisms by which a signal may be transmitted from the extracellular binding domain through the membrane to the intracellular signaling domain. All of the examples that are presented in the reference involve ways that cytoplasmic domains which are naturally attached to an extracellular binding domain receive the signal from the extracellular binding domain to become activated. However, the cytoplasmic domains of the instant invention are not naturally joined to an extracellular binding domain. Therefore, Models A, B and C do not apply to the present invention.

Regarding Model D, although this model describes intracellular domains which are not naturally joined to extracellular domains, this model is rejected by Applicants to apply to the claimed invention for the following reasons. Before turning to the rejection of this model as it applies to the present invention, Applicants need to clarify the Stoddard et al disclosure. Applicants respectfully disagree with the summary of model D set forth by the Examiner on page 3, paragraph 3. It is incorrect to characterize this model as requiring that a conformational change be a direct result of the physical contact of the ICs. Rather, as pointed out by Stoddard et al on page 2, column 2, second paragraph, the association of receptors occurs first and a second enzymatic step, eg., phosphorylation, causes a later conformational change. This downstream event is an identical process regardless of how the association is achieved. Thus, model D

does not require the association of two or more ICs to induce a conformational change in the ICs as proposed by the Examiner. In the instant invention, since the intracellular domains naturally interact with a variety of different proteins at the cytoplasmic surface, they are designed to be structurally flexible in their ability to associate. According to Kolanus et al (Cell 74: 171-183 (1993)), it is the clustering of cytoplasmic domains, such as zeta and the tyrosine kinases which are not naturally bound to extracellular domains, which is necessary and sufficient for the interaction of the intracellular domains with other proteins at the cytoplasmic surface and the activation of signaling.

The Kolanus reference is provided to show experimental support for clustering of the intracellular domains as the basic mechanism required for the signaling event. In contrast, the Stoddard reference only outlines potential requirements for a conformational change in these intracellular domains and is merely hypothetical. In re Marzocchi, 439 F2d 220, 169 USPQ 367 (CCPA 1975) states that an assertion by the USPTO questioning Applicants' enablement must be supported by either acceptable evidence or reasoning which substantiates the Patent Office's doubt of such enablement. Applicants submit that the reference supplied by the Pátent Office to question Applicants' enablement is merely hypothetical and does not provide the acceptable evidence required by the Marzocchi court. Accordingly, in view of the above rebuttal, withdrawal of this reference to support the 112 rejection should be removed.

Although Applicants have dismissed the Stoddard reference for its failure to apply to the instant invention for the reasons set forth above, Applicants would like to rebut each specific rejection raised by the Examiner using this

reference. In particular, Applicants traverse the rejection made on page 4 of the Office Action that the chimeric proteins of the instant invention are made of such disparate parts that "it is not deemed to be so predictable that the resulting chimeric proteins will have the three dimensional structure necessary to generate an intracellular signal according to any of the mechanisms set forth above." Applicants infer that this rejection implies that domains from different transmembrane signaling receptors, wherein each receptor may belong to any one of Models A-D described above, cannot associate to form functional recombinant chimeric receptors with any level of predictability. According to this logic, a chimeric receptor which contains a EC from a receptor which undergoes conformational changes and an IC which acts according to association mechanisms will be the least likely to successfully signal.

This logic is critically flawed since Applicants have prepared and demonstrated functionality of "the least likely to successfully signal" receptor, that being the CD4/zeta chimeric protein. Applicants' specification discloses a working example of the chimeric receptor containing mixed domains as described above, i.e., an extracellular domain derived from CD4, a receptor whose activity appears to depend on conformational changes (Stoddard et al page 6, "CD4 Domain 2", column 2, lines 17-21) and an intracellular domain derived from a protein whose activation is <u>not</u> dependent on conformational changes, but upon clustering or aggregation (Kolanus et al, Cell 74: 171-183 (1993)). (Upon binding of a ligand to the extracellular domain of the CD4/zeta chimeric receptor, transduction of a signal through the cytoplasmic domain occurs as measured by the activation of phosphitidylinositol and the tyrosine pathways, and the production of IL-2).

Further in the Office Action, it is stated that the instant specification exemplifies several different chimeric proteins, but does not show that all of these different constructed chimeric proteins are functional (page 4, paragraph 2). Applicants submit that the amount of enablement obtained from Applicants' disclosure present in the application and the knowledge of the art at the time of the claimed invention is sufficient for the skilled artisan to make and use the invention as it is mostly broadly claimed herein.

Applicants submit that the written description and the working examples disclosed in the specification demonstrate that the invention is sufficiently disclosed and enabled so as to allow one skilled in the art to practice the full breadth of the claimed invention without exercising undue experimentation. The number and variety of examples is irrelevant if the disclosures enabling and sets forth the "best mode contemplated". In re Borkowski et al. (CCPA 1970) 442 F2d 904, 164 USPQ 642. An applicant need not provide a specific example of everything embraced by a broad claim. In re Anderson (CCPA 1973) 471 F2d 1237, 176 USPQ 331.

Specifically, Applicants point out that more than one reproducible working example has been described in the instant application. Aside from the CD4/zeta example described above, the specification also includes other working examples, particularly the construction of a chimeric CD8/zeta receptor molecule and its activity upon expression in a transduced cell and the construction of a single chain antibody/zeta chimeric receptor (i.e., mouse anti-gp120 single chain antibody/zeta). Applicants have demonstrated the expression of this chimeric receptor on human T killer cells and the transduction of a signal (measured by cytolysis) upon the binding of specific

ligand to the extracellular domain of the chimeric receptor (See also Roberts *et al.*, Blood 84: 2878-2889 (1994)).

In addition, after the priority date that Applicants claim in the instant application, others in the field following Applicants' protocol have been successful in preparing chimeric proteins. For example, given the accessible materials and techniques well known at the time of the instant invention, Letourneur and Klausner (Science 255: 79-82 (1992)) demonstrated that the CD3 epsilon intracellular domain does function as well as zeta as a signalling domain in chimeric proteins. Moreover, other scientists have constructed CD16/zeta chimeric receptors and mouse anti-TNP single chain antibody/FcR gamma chimeric receptors and shown that both are capable of transducing cytolytic signals in T lymphocytes (Romeo *et al.*, Cell 68: 889-897 (1992); Eshar *et al.*, Proc. Natl. Acad. Sci. 90: 720-724 (1993)).

Furthermore, the examples in the specification describe extracellular domains, transmembrane domains and cytoplasmic domains from different proteins and from different species. For example, the specification discloses a single chain antibody/zeta chimeric receptor comprising an extracellular domain from a mouse antibody that is normally expressed in B cells, and a transmembrane domain from the human CD4 protein and a cytoplasmic domain from the human zeta chain that are normally expressed in T cells. Hence, the Examiner's argument that the instant claims should be limited to only the particular chimeric proteins tested because it would be unpredictable whether chimeric proteins encompassing disparate parts could initiate a signal has been clearly rebutted with sound legal and scientific arguments.

The Office Action further states on page 4, paragraph 4 that there is too much structural similarity in the various

domains chosen by Applicants as working examples of chimeric receptor proteins. Specifically, it is stated that chimeric receptors containing only Ig-like extracellular domains have been prepared. In rebuttal, Applicants draw the Examiner's attention to Letourneur and Klausner (*supra*) which describes the successful use of an extracellular domain from the Interleukin-2 Receptor, which is not Ig-like, in chimeric molecules of the instant invention. Further, on page 5, paragraph 1, the Examiner declares that all of the exemplified IC domains contain similar structural motifs. In rebuttal, Applicants draw the Examiner's attention to Kolanus et al (*supra*) which describes the successful use of the Syk tyrosine kinase, which does not contain similar motifs as zeta, as an intracellular domain in chimeric molecules of the instant invention. Next, in paragraph 2 on the same page, the Examiner questions whether IC domains from G-protein coupled receptors in particular would be operable in the claimed chimeric receptors. Applicants point out that the G-protein coupled receptors contain both a ligand-binding extracellular domain as well as an intracellular domain. Therefore, these intracellular domains are not part of the instant invention, since they are naturally joined to an extracellular binding-domain.

Applicants submit that the requirements for a functional chimeric receptor and the selection of the appropriate domains follow a fully defined and predictable protocol. An extracellular domain is selected solely based on its ability to bind another cell. The ligand need not be known and thus undue experimentation is not required. The transmembrane domain is selected based on its ability to span the plasma membrane and tested for its ability to function in the novel chimeric proteins. It does not matter whether the transmembrane domain is natural or synthetic, or derived from a multiple pass transmembrane protein or a single pass

transmembrane protein. The cytoplasmic domain is selected based on its ability to express an activation signal. These domains are spliced together using techniques well known to the skilled artisan.

It is well within the skilled artisan's ability to distinguish whether or not chimeric receptors are expressed on the host cell surface using techniques that are well standardized, e.g. immunoassays. The resulting chimeric receptors can be tested for cytolytic activity as described in the specification. Other routine screening methods for measuring activation well known in the art, including IL-2 production, Ca increase, activation of phoshitidylinositol, etc. may be used to identify functional vs. non-functional proteins. Thus, a person skilled in the art would be capable of constructing chimeric receptor proteins of the claimed invention, and would also be able to distinguish functional chimeric receptor proteins that transduce signals upon binding to the protein from non-functional proteins using routine screening.

Compliance under 35 U.S.C. Section 112, first paragraph may be met by combining the written description in the specification with additional evidence extraneous to the application's disclosure to determine the level and knowledge of one skilled in the art since sufficient enablement is evaluated by considering the disclosure in relation to the skill of one of ordinary skill in the art, and not to the general public. Standard Oil Co. v American Cyanamid Co., 227 USPQ 293, 297 (Fed. Cir. 1985).

The level of disclosure in the instant specification permits the skilled artisan to distinguish and avoid inoperative chimeric receptors. The knowledge of the recombinant molecular biology field at the time of the claimed

invention was such that given the amount of guidance described in the instant specification together with standard techniques available, one skilled in the art could construct chimeric receptors and measure effector function activity in a predictable fashion using routine screening without the exercise of undue experimentation. Applicants of the instant invention have satisfied the enablement requirement under section 112, by providing illustrative examples and a legally sufficient disclosure to support the breadth of the claims sought by Applicants. Any compositions that are inoperative that fall within Applicants' limitations of the generic claim are readily avoided by easy measurements that require no undue experimentation.

In summary, Applicants submit that the claimed invention meets the requirements of U.S.C. 112, first paragraph because Applicants' disclosure coupled with the state of the prior art readily permits the skilled artisan to reduce the claimed invention to practice without exercise of invention, ingenuity or undue experimentation. It is Applicants' position that the instant specification provides the required objective proof and the present disclosure provides an adequate teaching of the manner and the process of making and using the invention in terms which correspond to the scope of the claims sought to be patented. Accordingly, Applicants respectfully request that this rejection be withdrawn.

Rejection Under 35 U.S.C. 112, Second Paragraph

Claims 57-69 stand rejected under 35 U.S.C.§112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicants regard as their invention. Applicants have amended

the claims as follows to overcome the rejections raised by in the Office Action.

Claim 57 has now been amended to recite the novel chimeric protein "wherein said cytoplasmic domain is not naturally joined to an extracellular ligand binding domain" to avoid the redundant language that appears to be confusing. The removal of the replicative limitations avoided the need to add the word "binding" on line 10 of claim 57. Claims 62-63 and 66 have now been amended to recite "extracellular binding domain". The difference between claim 60 and 61 has now been clarified by the amendments to these claims. Claim 60 is now directed to a chimeric protein wherein the "extracellular binding domain is the heavy chain of an immunoglobulin or truncated portions thereof, containing ligand binding activity wherein said heavy chain of an immunoglobulin is by itself or in a protein complex with a light chain". This language was suggested by the examiner. In addition, claim 61 now recites that the chimeric protein wherein the extracellular binding domain is a single chain antibody or portion thereof both contain ligand binding activity. Claims 62 and 63 more distinctly claim Applicants' inventions by reciting that the extracellular domain is an "extracellular binding domain" and that it is "from" CD4 or CD8. Claim 66 has been further amended to recite that the mammalian cell containing a surface membrane protein wherein the extracellular binding domain is bound to a second protein to define a second ligand binding site. Support may be found, for example, on page 12 ,lines 25-29 in the specification. Claim 69 has been amended to recite "Major Histocompatiblity Complex antigens" to remove the abbreviation "MHC" and add the word "antigen" at the Examiner's request. Withdrawal of this rejection is respectfully requested.

Information Disclosure Statement

Accompanying this Amendment Applicants submit an Information Disclosure Statement and form PTO-1449, pursuant to 37 C.F.R. 1.56.

Conclusion

In view of the foregoing comments and amendment, it is believed that the subject application is in condition for allowance and notice to that effect is respectfully requested.

Any fees necessitated by the present filing may be charged to Applicants' deposit account as authorized in the transmittal letter submitted herewith in triplicate.

Respectfully submitted,

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